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Retention of eleven priority phenols using micellar electrokinetic chromatography

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ABSTRACT

The use of micellar electrokinetic chromatography (MECC) for the separation of eleven substituted phenols listed by the United States Environmental Protection Agency as priority pollutants was investigated. Solutions of potassium and sodium dodecyl sulphate in phosphate–borate buffer of pH 6.6, 7.0 and 7.5 were used as the electrophoretic media. Satisfactory separation of the eleven phenols was obtained using a 180-μm capillary at 10 kV and pH 6.6 with a solution containing both sodium and potassium dodecyl sulphate. Observations on the retention behaviour of the phenols in MECC were related to their physico-chemical properties.

INTRODUCTION

Capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MECC) have become popular separation techniques owing to their inherently high separation efficiency[1–4]. The analysis of a series of chlorophenols using MECC has been reported[5]. However, the application of these techniques to the analysis of environmental pollutants has rarely been investigated.

Substituted phenols are of great environmental concern owing to their high toxicity. The United States Environmental Protection Agency (USEPA) lists the eleven phenols shown in Fig. 1 as priority pollutants[6]. Some of these phenols, which originate from such diverse sources as pesticide application, industrial wastes, water supplies and automobile exhausts, are highly toxic even at low concentrations. A widely used technique for the analysis of phenols is high-performance liquid chromatography (HPLC) with either reversed-phase isocratic or gradient elution[7–10]. However, owing to the inherent limited resolving power of conventional HPLC techniques, optimization of the separation of the phenols often involves complicated procedures or a large number of experiments[11]. In this work, the use of MECC for the separation of the eleven priority phenols was investigated. For this purpose, two types of micellar solutions were used to examine the selectivity. The retention behaviour of these compounds in MECC at various pH values is discussed.

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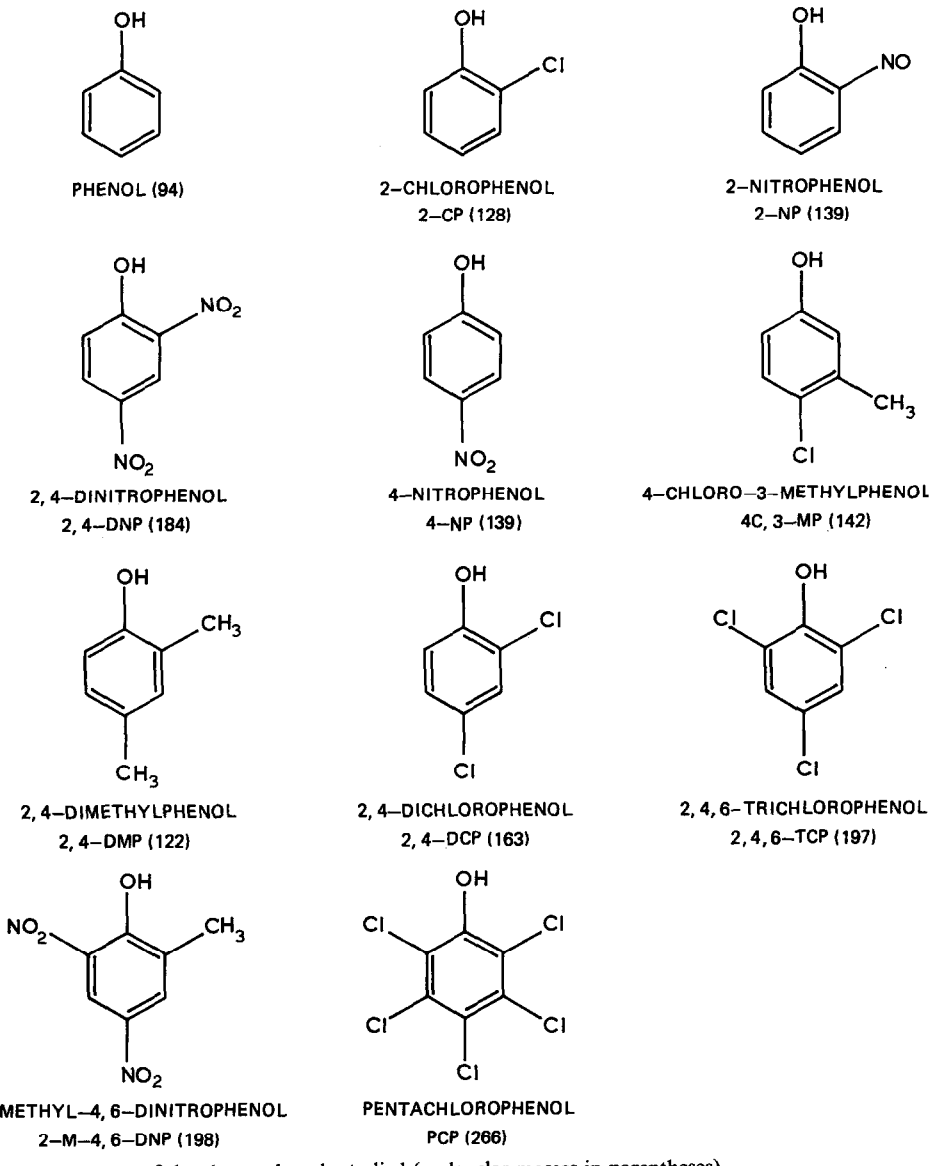

Fig. 1. Structures of the eleven phenols studied (molecular masses in parentheses).

EXPERIMENTAL

The experiments were performed on a laboratory-built MECC system. A Spellman (Plainview, NY, U.S.A.) Model RM15P10KD (15 kV maximum) power supply was used to maintain the high voltage. The columns used were fused-silica capillary tubes of 180 and 50 μm I.D. (J & W Scientific, Folsom, CA, U.S.A.) with effective lengths of 1 and 0.85 m, respectively. Peaks were detected with a micro-UV–VIS

detector (Carlo Erba, Milan, Italy) with the wavelength set at 254 nm. The detection cell for on-column detection was formed by removing the polyimide coating of a small section of the fused-silica tubing used for electrophoresis.

Chromatographic data were collected and analysed on a Hewlett Packard (Palo Alto, CA, U.S.A.) Model 3390A integrator. A Linear Instruments (Irvine, CA, U.S.A.) Model 252A/MM chart recorder was also used to record the chromatograms. Samples were introduced manually by gravity feed: sample solution was introduced into one end of the capillary tube by siphoning from sample solution at a level higher then that of the electrophoretic solution in which the other end of the tube was immersed. An injection time of 5 s at a height difference of 5 cm was used. A schematic diagram of the experimental set-up is shown in Fig. 2.

Two types of electrophoretic media were used. Both were phosphate–borate buffer solutions prepared as described previously[5]. For the solution containing only sodium dodecyl sulphate (SDS), sodium dihydrogenphosphate and sodium tetraborate were used, whereas for the solution containing potassium dodecyl sulphate (KDS), potassium dihydrogenphosphate and sodium tetraborate was used in preparing the buffer. For brevity, the two solutions are henceforth referred to as solutions 1 and 2, respectively. In both instances the pH of the buffer solution was adjusted by mixing phosphate and borate in an appropriate ratio. The pH was measured using a Hanna (Limena, Italy) Model H18417 pH meter. SDS of analytical-reagent grade was dissolved in the buffer. For solution 2, as the Kraff point of KDS is higher than room temperature, precipitation of some of the KDS would be expected. The solution was therefore filtered before use to remove any precipitate formed during mixing. A sample mixture of the eleven priority phenols and Sudan III was prepared in methanol. The concentration of each phenol in the sample mixture was 1000 ppm. Sudan III was used to obtain the retention time of the micelles and its concentration was 100 ppm.

RESULTS AND DISCUSSION

Preliminary experiments using the conditions listed in Table I were performed with the two types of solutions at pH 6.6 and at a voltage of either 10 or 15 kV for the

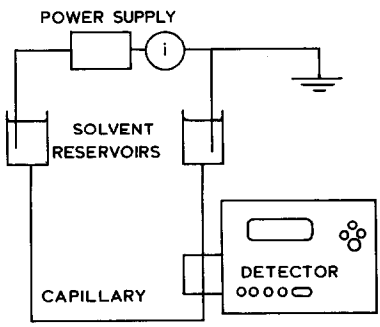

Fig. 2. Schematic diagram of MECC instrument.

TABLE I

EXPERIMENTAL CONDITIONS FOR THE PRELIMINARY EXPERIMENTS AND THE t_0, t_{mc}, t_0/t_{mc} AND v_{eo} VALUES OBTAINED

Parameter	*Experiment 1*	*Experiment 2*	*Experiment 3*	*Experiment 4*
Tubing I.D. (μm)	180	180	50	50
Electrophoretic solution	1	2	1	2
Power (kV)	10	10	15	15
Current (μA)	54	130	7.2	15
t_o (min)	12.6	9.0	9.6	15.3
t_{mc} (min)	37.2	45.6	36.7	41.5
t_o/t_{mc}	0.34	0.20	0.26	0.36
v_{eo} (mm s^{-1})	1.32	1.85	1.48	0.93

180- and 50-μm I.D. capillary tubings. The results obtained are listed in Table II. The apparent capacity factors k' were calculated according to the equation[5]:

$$k' = \frac{t_R - t_0}{t_0 [1 - (t_R/t_{mc})]} \quad (1)$$

were t_R, t_0 and t_{mc} are the elution times of the solute, the insolubilized solute and the micelle, respectively. In the experiments, t_0 was regarded as the retention time of methanol and t_{mc} that of Sudan III.

From the results in Table II, it can be seen that among the four sets of conditions, the combination of the 180-μm I.D. capillary tubing with solution 2 (experiment 2) provides superior selectivity for the phenols. In fact, all eleven phenols were satisfactorily separated using this set of conditions. The variation in selectivity could be explained in terms of the t_0/t_{mc} ratio and the electroosmotic velocity, v_{eo}. The

TABLE II

CAPACITY FACTORS OF THE PHENOLS AT pH 6.6 USING THE EXPERIMENTAL CONDITIONS IN TABLE I

No.	*Solute*	*Capacity factor*			
		Experiment 1	*Experiment 2*	*Experiment 3*	*Experiment 4*
2	4-NP	0.95	2.76	0.20	0.05
3	2,4-DCP	1.15	3.23.	0.22	0.08
4	2-NP	0.95	3.76	0.33	0.15
5	2-CP	1.15	4.67	0.34	0.18
6	TCP	1.15	6.82	0.35	0.22
7	PCP	1.22	8.98	0.44	0.22
8	DNOC	1.22	10.39	0.44	0.83
9	2,4-DNP	1.70	11.64	0.44	1.25
10	Phenol	0.83	13.11	0.18	1.47
11	4C,3-MP	1.70	40.72	0.47	1.47
12	2,4-DMP	2.55	46.44	0.88	8.27

values of t_0/t_{mc} and v_{eo} obtained for the four experiments are included in Table I. As shown by Otsuka *et al.*[12], a smaller t_0/t_{mc} ratio would result in better resolution between peaks. From Table I, it can be seen that the t_0/t_{mc} ratio obtained for experiment 2 was the smallest among the four sets of experiments. Consequently, better separation and selectivity could be obtained using this set of conditions. Another reason for the difference in selectivity could be attributed to the v_{eo} values. As shown by the results of Terabe *et al.*[13], obtained for a range of v_{eo} values up to 2 mm/s, the HETP for most of the phenols decreased with increasing v_{eo} in the range studied. Similar results were obtained in this work, as is evident by the large v_{eo} value obtained for experiment 2 which favours better separations.

Another observation was that the capacity factors calculated using eqn. 1 for experiment 2 were much larger than those for the other experiments. This could be due to the difference in the t_0 values obtained for each set of experiments. As indicated in Table I, the t_0 value obtained for experiment 2 was the smallest among the four experiments. From eqn. 1, it can be seen that a small t_0 would result in larger capacity factors. This is in agreement with the results obtained.

It is also worth noting that with different solutions slight changes in the retention order were observed. One notable example is the retention order for phenol. In experiments 1 and 3, the retention order seems to be similar to that obtained by Terabe and co-workers.[3,5] However, in the other two instances, the phenol peak was observed much later. The reason could be that even though the fused-silica capillary tubing was deactivated, there is a possibility that some free OH groups are not completely removed. These free OH groups would be susceptible to the formation of hydrogen bonds with solutes having suitable substituent groups. As a result, these solutes would be retained much longer in the column. Among the eleven compounds, only phenol seems to be substantially affected. Consequently, phenol was retained much longer despite its low hydrophobicity. On the other hand, for most of the larger phenols, such as PCP and TCP, because of steric hindrance by the substituent groups, formation of hydrogen bonds with the free OH groups is not favourable. With solu-

TABLE III

CAPACITY FACTORS OF THE PHENOLS AT pH 7.0 AND 7.5 AND THEIR LOG *P* AND pK_a VALUES USING THE 180-μm I.D. FUSED-CAPILLARY TUBING AND SOLUTION 2

Solute	*log P*	pK_a	*Capacity factor*	
			pH 7.0	*pH 7.5*
4-NP	1.96	7.16	0.359	0.310
2,4-DCP	3.08	7.89	1.838	1.100
2-NP	1.79	7.23	0.083	0.107
2-CP	2.15	8.55	0.083	0.107
TCP	3.77	6.23	1.838	1.174
PCP	5.85	4.50	1.838	0.711
DNOC	2.77	4.70	0.841	0.711
2,4-DNP	2.29	4.07	0.355	0.447
Phenol	1.46	11.00	0.841	0.711
4C,3-MP	2.95	9.54	1.838	1.100
2,4-DMP	2.42	10.59	0.841	0.711

tion 1, as no precipitation occurs, the higher surfactant concentration is probably able to protect the phenol molecules from interaction with the free OH groups on the silica tubing. Hence, phenol was eluted in the usual retention order, which is governed by the hydrophobicity.

As experiment 2 provided better selectivity, subsequent experiments were carried out using this set of conditions; pH 7 and 7.5 were also investigated. The results obtained and the pK_a and log P values for the phenols are given in Table III. As pH 6.6 gave the best separation, the order of elution at this pH was used as the reference for discussion. A typical electropherogram obtained using pH 6.6 is shown in Fig. 3. The eleven compounds were divided into three groups on the basis of their retention characteristics. The apparent capacity factors for the eleven phenols are plotted in Fig. 4, which illustrates that in general the capacity factors for pH 6.6 were much larger than those at higher pH. It was noted that compounds in group I (peaks 2–5) have fairly high pK_a values (6.23–8.55) and therefore at pH 6.6 these compounds would either be neutral or partially ionized. For the compounds in group II (peaks 7–9), the pK_a values were smaller and these compounds would be expected to be completely ionized at pH 6.6. Therefore, the compounds in group II would be less solubilized by the micelles and the capacity factors should be smaller than those of group I. However, the opposite trend was observed. The reason could be that the compounds in group II, being ionized and thus negatively charged, would have a tendency to migrate to the positive electrode (electrophoretic flow). Owing to this electrophoretic pull in the direction opposite to the electroosmotic flow, group II compounds reached the detector at relatively longer times than those of group I and

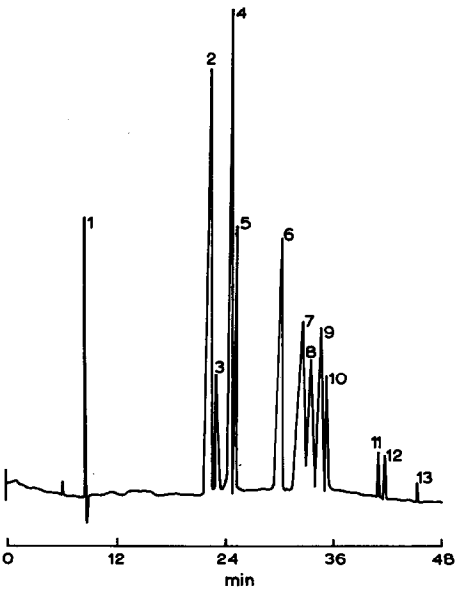

Fig. 3. Electrokinetic chromatogram of the eleven phenols with solution 2. Buffer, SDS (0.05 *M*) in phosphate (0.005 *M*)–borate (0.01 *M*); pH, 6.6; separation tube, 1 m × 180 μm I.D. fused-silica capillary; voltage, 10 kV; current, 130 μA; detector wavelength, 254 nm. Peaks: 1 = methanol; 13 = Sudan III; other peak numbers as in Table II.

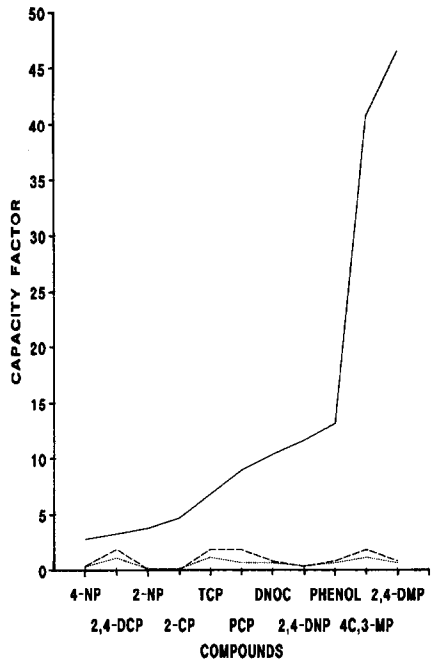

Fig. 4. Variation of capacity factors of phenols with pH. Solid line, pH 6;6; darked line, pH 7.0; dotted line, pH 7.5.

hence have higher capacity factors. The compounds in group III (peaks 10–12), having fairly high pK_a values, are in the neutral form and therefore, they would be solubilized more easily by the micelles. It was also noted that the two compounds that were eluted last (4-chloro-3-methylphenol and 2,4-dimethylphenol) contain one or two methyl groups. This may account for the fact that the two compounds have exceptionally high capacity factors, as SDS and KDS contain long alkyl chains and their interaction with the methyl groups on the two compounds would be stronger, tending to enhance solubilization.

From the results in Table III, it was found that for pH 7.0 and 7.5, the trend in the capacity factors appeared to be governed by the trend in the partition coefficients, log P, *i.e.*, an increase in capacity factor with increase in log P was observed. The exceptions to the general trend are phenol and pentachlorophenol. Phenol has a small size and hence a greater tendency for partition with the micelles. Further, because of the possibility of forming hydrogen bonds with the free OH groups on the silica tubing, its capacity factor was found to be exceptionally high. With pentachlorophenol, which has a large log P value but a small pK_a, there is a greater tendency for it to be ionized and thus less solubilized by the micelles. Despite the high log P value, its capacity is lower than those of 2,4-dichlorophenol and trichlorophenol.

In Fig. 4, it is also noted that in general the capacity factors decrease with increase in pH. The differences in capacity factors between the eleven phenols were not very significant at pH 7 and 7.5, whereas larger differences were observed at pH 6.6. The reason is that as the pH increases, more of the phenols would ionize to the

anionic form, resulting in a higher electrostatic repulsion between the ionized solutes and the SDS micelles. Despite the increased interaction of the anions with the positive electrode, the effect of the stronger electrostatic repulsion with the micelles dominates, which tends to suppress micellar solubilization. Consequently the net effect is that the capacity factors decrease at higher pH. As the capacity factors for all the phenols are small under these conditions, the differences between them are also relatively small. This observation is in agreement with that of Otsuka *et al.*[5] At pH 6.6 the differences were more obvious because at this pH most neutral compounds are highly solubilized by micelles. Hence they would be retained for a longer time and larger capacity factors and greater differences were observed.

The results obtained show that MECC is a relatively simple yet powerful technique that can be used for the analysis of priority phenols. To achieve satisfactory separation in this study, a very simple instrument was used, *i.e.*, the high-pressure pumps and chromatographic columns normally used for HPLC were not required. Satisfactory separation of a relatively complicated mixture can be easily achieved owing to the inherently high resolving power of the technique. Therefore, further investigations on the use of capillary electrophoretic techniques for the analysis of environmental pollutants would be an interesting area of development.

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REFERENCES

1 F. E. P. Mikkers, F. M. Everaerts and Th. P. E. M. Verheggen, *J. Chromatogr.*, 169 (1979) 11.
2 J. W. Jorgenson and K. D. Lukacs, *Anal. Chem.*, 53 (1981) 1298.
3 S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, *Anal. Chem.*, 56 (1984) 111.
4 S. Terabe, K. Otsuka and T. Ando, *Anal. Chem.*, 57 (1985) 834.
5 K. Otsuka, S. Terabe and T. Ando, *J. Chromatogr.*, 348 (1985) 39.
6 *Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants*, U.S. Environmental Protection Agency, Environment Monitoring and Support Laboratory, Cinicinnati, OH, 1977.
7 N. G. Buckman, J. O. Hill, R. J. Magee and M. J. McCormick, *J. Chromatogr.*, 284 (1984) 441.
8 F. P. Bigely and R. L. Grob, *J. Chromatogr.*, 350 (1985) 407.
9 S. F. Y. Li and H. K. Lee, *Chromatographia*, 25 (1988) 515.
10 K. Abrahamsson and T. M. Xie, *J. Chromatogr.*, 279 (1983) 199.
11 C. P. Ong, H. K. Lee and S. F. Y. Li, *J. Chromatogr.*, 464 (1989) 405.
12 K. Otsuka, S. Terabe and T. Ando, *J. Chromatogr.*, 332 (1985) 219.
13 S. Terabe, K. Otsuka and T. Ando, *Anal. Chem.*, 61 (1989) 251.